most powerful action, phenol the least, while methylene blue and formaldehyd exerted an equal influence intermediate between the other two.

The presence of native protein of inflammatory cerebrospinal fluid did not interfere with the inhibitory action of methylene blue on the meningococci.

The selective inhibitory action of methylene blue on the growth of the meningococcus group of organisms may be of a specific chemical nature, or it may be nonspecific, and due to the fact that the meningococcus is a relatively frail organism requiring, when artificially cultivated, a fairly congenial environment. To determine the specificity of the reaction would require the use of a greater variety of structurally allied chemicals and the titration of each against a variety of bacterial suspensions.

This work was approached rather from the practical point of view and an inquiry into the nature of the inhibitory phenomenon has not been attempted.

Contact with any considerable number of cases of epidemic cerebrospinal meningitis emphasized the fact that serum treatment as commonly practiced leaves much to be desired. Under ideal conditions, that is, early treatment with an effective serum, results are of course of proved success. But under conditions of late diagnosis and treatment with serum obtained from presumably reliable sources, but often of little or no value, results are frequently discouraging.

The hope of finding a chemical therapeutic agent for the meningococcus group of infections is what prompted this study. Methylene blue may possibly fill the requirements. It inhibits the growth of meningococci in fairly high dilutions. It is relatively nontoxic, is said to be diffusible through the choroid plexus and to have a specific affinity for nerve tissue.

Application to in vivo conditions from experiments conducted in vitro must, of course, be made with caution.

THE DISSEMINATION AND DESTRUCTION OF TYPHOID BACILLI INJECTED INTRAVENOUSLY IN NORMAL AND IMMUNE RABBITS

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Typhoid fever, although one of the first diseases in which the causative factor, the typhoid bacillus, was found, is still among the diseases which rank high in yearly mortality and disability statistics. This is especially true in those places where the value of prophylactic immunization is not widely advertised, or where owing either to carelessness or ignorance, those attending typhoid cases or convalescents do not take proper precautions against infection from their patients. However, in those countries where vaccination is advised, or, as in the case of the American and British armies, compulsory, typhoid fever has dropped to a level where it is no longer considered a serious menace to the population. Next to the vaccination against smallpox, typhoid vaccination has proved the most successful in preventing disease, although the duration of the immunity is not as long as that conferred by smallpox vaccination. But in spite of our early knowledge of the localization and characteristic of the typhoid bacillus, and our present knowledge of the immunity either on recovery from the disease itself or by vaccination, the exact mechanism by which the body is able to protect itself and the manner in which it gets rid of the offending invaders, is still unknown. Could we but understand the exact nature of this mechanism in a disease such as typhoid where our knowledge of the organism, symptoms, and such are so clear, we might well be able to apply similar deductions to other diseases in which the cause is more or less obscure, and undoubtedly assist the body in its fight to overcome the abnormal conditions under which it is struggling to maintain its own.

The typhoid bacillus was first demonstrated, as is well known, in 1880. Eberth, Klebs, and Koch simultaneously reported the finding of small rods in the tissues of those dying of typhoid fever, and in a sufficient number of cases to warrant their suspicions that this was the causative factor of the disease. However, Eberth[1] is usually given credit for the discovery because of

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[1] Virchows Arch., 1880, 81, p. 58.

a slight priority in the appearance of his article. The organism was first grown on artificial culture medium by Gaffky[2] in 1884. Klebs, just previous to the article by Gaffky, had grown cultures of organisms from the tissues of cases succumbing to typhoid, but his description of his cultures is considerably at variance with the typhoid bacillus as it is generally pictured in later and more conclusive work. However, the chains of organisms which he describes may have been due either to some peculiarity in the medium used, or to the fact that organisms recently isolated from the host may have certain unusual appearances which they lose after artificial cultivation. In 1885 A. Pfeiffer[3] isolated the bacillus from stools of typhoid patients and in 1886 Heuppe demonstrated the organisms in the urine. In 1886 Fraenkel and Simmonds[4] isolated the organism from the blood of a cadaver, but in 1887 came the important discovery of Vilchur, who was successful in isolating typhoid from the blood of living patients, although his percentage of positive cultures was very small.

In 1891, Blackstein[5] reported the important discovery that the bile of rabbits injected intravenously with living typhoid bacilli, harbored these organisms for as long as 109 days after inoculation. Fütterer[6] had previously laid stress on the fact that typhoid bacilli were found in the bile of cases of fever coming to necropsy, a finding which disproved, to a certain extent, the former belief that the bile was of high antiseptic value. The report of Blackstein that he had isolated the bacillus from the gallbladder 109 days after inoculation, however, was of far greater importance since it opened up a new avenue for study of a disease which, at that time, was making great inroads on the population. The route by which the bacillus reached the gallbladder was immediately subject to investigation. The theory that it ascended from the intestine was the first to be brought forth, but this was soon cast aside in favor of two more logical theories, namely, (1) that the organism is brought to the liver by the portal circulation, and from there swept down with the bile into the gallbladder and thence into the intestine, or (2) that it is carried directly to the gallbladder by the blood vessels which supply it. The former theory has been supported by the experimental work of Doerr,[7] Nichols,[8] and others, while the latter theory has been substantiated by the work of J. Koch,[9] Chirolanza,[10] Forster and Kayser.[11] Chiari[12] thinks all three ways possible. But the work of Blumenthal[13] and later investigators would seem to prove that the latter theories are the more logical. As examples of the work to substantiate these two theories we may give a brief outline of the experiments carried out by Doerr and Chirolanza. Doerr, for example, ligated the common duct of rabbits, after which they were given intravenous injections of living typhoid bacilli. Gallbladder infection was, in these animals, constant. If, on the other hand, the cystic duct was ligated, he could demonstrate no organisms in the gallbladder. Chirolanza found typhoid bacilli present in the gallbladder

[2] Mitt. a. d. Kais. Gesundheit, 1884, 2, p. 372.
[3] Deutsch. med. Wchnschr., 1885, 12, p. 500.
[4] Die Aetiologische Bedeutung des Typhus-bacillus, 1886.
[5] Bull. Johns Hopkins Hosp., 1891, p. 96.
[6] München. med. Wchnschr., 1888, 35, p. 315.
[7] Centralbl. f. Bakteriol., 1905, 39, p. 624.
[8] Jour. Exper. Med., 1914, 20, p. 573.
[9] Ztschr. f. Hyg. u. Infektionskr., 1909, 62, p. 1.
[10] Ztschr. f. Hyg. u. Infektionskr., 1900, 62, p. 11.
[11] München. med. Wchnschr., 1905, 52, p. 1423.
[12] Centralbl. f. Bakteriol., 1894, 15, p. 648.
[13] Centralbl. f. Bakteriol., I, O., 1910, 28, p. 1314.

when the cystic duct was closed. He pointed out, furthermore, that the gallbladder is well supplied with blood vessels and that typhoid bacilli were sometimes found in the folds of the mucosa near the capillaries, whereas the bile itself might be sterile. J. Koch was also able to demonstrate these clumps of organisms in cut sections. No matter what theory may be assumed to be correct, the fact still remains that typhoid bacilli can be isolated from the gallbladder for a long period of time after injection. For example, Chirolanza demonstrated them 58 days after injection, Blackstein—109 days, Morgan[14]—4 months, and Uhlenhuth and Messerschmidt[15]—6 months. Doerr was able to isolate it from the interstitial mucosa 120 days after inoculation, which probably meant a locus of infection in the gallbladder, from which organisms were being swept down with the bile.

The establishing of "carrier" rabbits has not been successful in the hands of all investigators. Blackstein, for instance, could obtain only about 50 per cent. of carriers in his inoculated animals, and Nichols,[16] in his earlier experiments, could not produce this condition with any degree of certainty. However, Doerr could get fairly high percentages of carriers, as could Gay and Claypole,[17] and it would appear from later writings that Nichols himself was finally successful. In fact, it is now certain that if the proper technic is employed, the establishing of carriers is an easy matter. Various methods of injecting the culture have been and still are employed, some favoring direct injection into the gallbladder—(Hailer and Rimpau)[18] and others injection into the mesenteric vein (Nichols). But ordinarily the marginal vein of the ear is used, as it proves quite satisfactory and is easy of access.

It was not long after the discovery of the gallbladder localization of typhoid bacilli, that attention was drawn to the danger that might result from the spread of infection either by healthy carriers or by convalescents, by means of stools and urine. Petruschy,[19] in 1898, reported a case in which there was direct evidence that a healthy attendant had been infected by the urine of a convalescent. R. Koch,[20] however, was one of the first to emphasize the importance of the close observation of convalescents and carriers and advocated the establishment of research stations in those parts of the country in which typhoid fever was prevalent. It was his idea to carefully examine the stools and urine of all suspected carriers and convalescents and to safeguard other people as much as possible. The importance of detecting these carriers may be realized when one examines the reports of various investigators on the percentage of carriers that they have been able to detect. A chronological summary of the percentage of carriers reported by different authorities during approximately the last 10 years, is contained in a recent treatise on typhoid fever by Gay.[21] Taking the average of these figures, we find that 4.3 per cent. gives a reasonable basis on which to judge our carrier percentage. That is to say, of every 100 cases of definitely proven fever, four or over harbor and eliminate typhoid bacilli for 3 months or more after recovery from the disease. Adding to these those persons who are carriers without any history of typhoid fever, it is very probable that the number is in reality greater than is normally estimated. It is this latter type, namely, the healthy "carrier" that is especially liable to be

[14] Jour. Hyg., 1911, 7, p. 202.
[15] Deutsch. med. Wchnschr., 1912, 38, p. 2397.
[16] Jour. Exper. Med., 1916, 24, p. 497.
[17] Arch. Int. Med., 1913, 12, p. 613.
[18] Arbeit. a. d. Kais. Gesundh., 1912, 36, p. 409.
[19] Centralbl. f. Bakteriol., 1898, 23, p. 577.
[20] Veröffentlichungen A. D. Militärsanitätswegen, 1092, 21.
[21] Typhoid Fever Considered As a Problem of Scientific Medicine, 1918.

overlooked and consequently an important factor in the spread of infection through the handling of foodstuffs, especially milk and the like. The isolation of typhoid bacilli from the feces of such persons may not always be easy. Some carriers seem to eliminate the organisms almost continually, whereas with others, this elimination appears to be spasmodic. In certain suspected carriers where daily cultures have given negative results, purgatives such as elaterin, etc., are given to bring down material from the duodenum. However, animal experimentation has shown that gallbladder infection may still be active, although platings of intestinal contents are negative.

It is most natural that attempts to cure this condition should follow close in the wake of its discovery. One of the first put forth was that suggested by Forster,[22] namely the administration of bile salts or dried bile. By this he hoped to increase the secretion of bile and thereby to wash out the organisms from the gallbladder. In 1910, Tsuzuki and Ishida[23] reported on some cases treated with iodin in the form of potassium iodid, in conjunction with Fowler's solution, with promising results, basing their opinion on the fact that the excreta of persons so treated cleared up more quickly than those of untreated cases. Conradi[24] used rectal injections of chloroform mixed with milk and cream and claimed very satisfactory results with laboratory animals. Knick and Pringsheim[25] found that the bile of dogs treated per os with large doses of menthol, methylene, hippuric acid and urotropin, was found to be bactericidal or strongly inhibitive to the growth of B. typhosus in broth. Mercurous chlorid, sodium salicylate, salicylic acid, oil of turpentine and methylene blue had little or no effect. Uhlenhuth and Messerschmidt[15] tried monochlor-acetylcholic acid, salicylate of copper, arsphenamin, phosphorus and colloidal mercury with negative results . Hailer and Rimpau[18] injected rectally methyl iodid, ethylbromid, chloroform, and iodoform in chloroform, all of these mixed with milk and cream before injection. They found the first two substances too toxic for use in laboratory animals, but reported occasional success with the use of iodoform in chloroform and also with bromoform, but do not recommend any of these for practical use. Kalberlah[27] gave his carriers (human) tincture of iodin in conjuction with charcoal and cites apparently cured cases, if one is justified in judging from repeated negative plates. Hailer and Wolf[28] tried phenols and ethereal oils, using xylol, thymol, and pyrogallol as representatives of the former and sandalwood oil, pinene, eucalyptol, and cinnamon oil, of the latter. It was only with the last named substance that they had any encouraging results. Nichols[29] in 1917 proposed an alkaline treatment of early gallbladder carriers. He suggests rendering the bile more alkaline by the administration of sodium bicarbonate, since bile with an increased alkalinity is destructive to typhoid bacilli. He was able to demonstrate an actual increase in the alkalinity of the bile after the administration of sodium bicarbonate and by such treatment was able to clear up several carriers. The treatment suggested by Hertz[30] may be mentioned here, namely, the injection of protein (milk) into the gluteal muscle. This causes a temperature rise, chill, and elimination of organisms and is claimed by the author to have been successful in several instances.

[22] Verhandlung der Deutschen Patholog. Gesellschaft, 1907.
[23] Deutsch. med. Wchnschr., 1910, 36, p. 1005.
[24] Ztschr. f. Immunitätsforsch., 1910, 7, p. 158.
[25] Deutsch. Arch. klin. Med., 1911, 101, p. 137.
[27] Med. Klin., 1915, 11, p. 581
[28] Arbeit. a. d. Kais. Gesundh., 1915, 48, p. 80.
[29] Jour. Am. Med. Assn., 1917, 68, p. 958.
[30] Wein. klin. Wchnschr., 1916, 29, p. 1290.

Soon after the value of prophylactic vaccination became established, vaccine therapy as a means of clearing up carriers was inaugurated and for a time considered successful. R. Koch[30] as early as 1902 suggested this vaccine treatment of the carrier. Irwin and Houston[31] reported a typhoid carrier cured by the administration of an autogenous vaccine and Meader[32] in 1910 likewise reported a case successfully treated. Following these many other apparent cures were reported and for a time this method of treatment was thought to promise a solution of the carrier problem. Johnston[33] reported some work on experimental rabbit carriers and claimed success in vaccine treatment of these. But certain of his results are so peculiar and divergent from those obtained by Doerr,[7] Bull,[34] Francke and Parker,[35] and others as well as in this laboratory, that one must question the validity of his results. For instance, he reports that in normal animals positive blood cultures appear in from 7-10 days after intravenous inoculation, and that the blood did not become sterile for from 30-60 days. In vaccinated animals the blood cultures were sterile in from 35-45 days after inoculation. In similar work on the same problem by the above authors, the blood cultures in normal animals became positive immediately on inoculation, and remained so constantly for from 10-14 days. Later than this it was rarely possible to get a positive blood culture.

Whatever may have been the apparent success of these earlier experimenters, repetition of this work and subsequent use of vaccine both in human and animal carriers have given little more than negative results. In fact, this method of treatment has been practically discarded and those who are seeking the solution of the carrier problem have been forced to turn again to the use of chemicals whose introduction into the body may be fatal to the organism but harmless to the host.

We have recently, in this laboratory, carried out a few experiments on the carrier rabbit using dyes which were known to be excreted through or broken down by the liver in hopes that their action on the organisms there localized might be powerful enough to cause their complete destruction. Safranin, methyl violet, crystal violet, ethyl violet, and Spiller's purple were the principal dyes used, but the results were entirely negative. Rabbits showed very little tolerance for most of these dyes and especially for methyl violet, indicating that these are unsuitable for this type of therapy and that further work along this line will have to be done with chemicals less toxic for experimental animals.

Knowing what we do about the typhoid bacillus, its entry into the body, localization in the various organs, and ultimate elimination from these organs, two points of interest suggest themselves to the investigator. First, is there any difference between the normal and immune animal, in the length of time that elapses before any or all organs become sterile after intravenous injection of living typhoid bacilli? And secondly, if there is any difference, just what is the mechanism involved and what factor or factors are responsible for this

[31] Lancet, 1909, Jan. 30, 1909, Vol. 115.
[32] Bull. Johns Hopkins Hosp., 1910.
[33] Jour. Med. Research, 1912, 27, p. 177.
[34] Jour. Exper. Med., 1915, 22, p. 475; ibid., 1916, 23, p. 419.
[35] Jour. Med. Research, 1919, 39, p. 301.

ability of the immune rabbit to so quickly rid itself of large numbers of living organisms? Typhoid, as we know, is a bacteremia, and therefore the bacilli must be carried to all parts of the body by the circulating blood. The question arises as to whether the organisms are killed off simultaneously in the blood and tissues, or remain in the organs after blood cultures are negative.

Doerr was the first to touch on this first phase of the mechanism of resistance and made cultures from the liver, spleen, blood and marrow of rabbits previously inoculated intravenously with living bacilli. In his experiments, carried out on normal animals alone, he found a rapid elimination of the organisms by all of the organs, and after the 14th day could not isolate them from the blood or any of the viscera, except the gallbladder. No attempts to determine the relative number of bacilli per given weight of organ were made, positive or negative results being based on the streaking of plates with the cut surfaces of organs or on the culturing of small pieces of organs in broth. Bull has also done considerable work on tracing the organisms shortly after intravenous injection, particularly as to their disappearance from the blood. He used only a small number of organisms ($\frac{1}{35}$-$\frac{1}{40}$ agar slant) and his findings were those taken from 1 minute to one-half hour after injection and with normal rabbits alone. He found that the bacilli disappear very rapidly from the organs and especially from the circulating blood so that when $\frac{1}{40}$ of a culture of B. typhosus was injected, in 15 minutes only 1 organism per c c of blood could be isolated. Bartlett has worked on the intravenous inoculation of dogs with Micrococcus aureus and found that the organisms were rapidly taken up particularly by the liver and spleen, but that these same organs were the first to become sterile. Recently, the work of Parker and Francke, on the fate of intravenously injected typhoid bacilli into normal and immune rabbits, appeared. The technic used in estimating the number of bacteria per given weight of organ was similar to that used in experiments on the same problem described in this paper, with the exception that the number of bacteria injected in our experiments was probably smaller and the observations extended over a longer period of time. The authors mentioned found large numbers of bacilli in the organs of their animals and could demonstrate their rapid decrease, but concluded that there was little or no difference in the bactericidal properties of normal and immune rabbits. It would seem, however, that too many organisms were injected and

observations made over too short a period of time to fully warrant the conclusions drawn by them, conclusions which are not verified by the experiments described in this paper.

The second point which comes to mind is somewhat more difficult of approach. It is, namely, the mechanism involved in this ability of the body or body cells to rid themselves of the offending organisms. In other words, just what tissue or tissues are responsible for or take part in the restoring of the body to normal conditions. Is it the blood or tissue cells, or is it an interaction of these two that is responsible? Bull believes that the bacteria are clumped in the blood stream and accumulate in the capillaries of the organs especially the liver and spleen, where they are ingested by the polymorphonuclear leukocytes and destroyed. But this does not explain why the blood of immune rabbits is nonbactericidal even when large numbers of leukocytes are present or artificially introduced. Johnston claims that the bactericidal properties of normal serum for typhoid bacilli are practically nonexistent, whereas these properties are increased by vaccination. But the work of Bull, Teague, Buxton and others, and our own experiments would seem to prove the contrary to be true, namely, that normal serum is highly bactericidal to typhoid bacilli, whereas this power in highly immunized animals is practically nil.

THE FATE OF TYPHOID BACILLI IN NORMAL AND IMMUNE RABBITS

Normal Rabbits.—The question of the distribution and destruction of large numbers of living typhoid bacilli which have been injected intravenously into normal rabbits must be solved by determinations extending over weeks rather than minutes. In the following tabulated experiments care was taken that the number of organisms injected was as uniform as possible. A technic similar to that of Gay and Claypole, by which they were able to produce a high percentage of carriers, was followed.

Tubes of definite diameter (1.8 cm.) were used, containing 10 per cent. blood agar and slanted on an especially constructed tray, so that the slant extended almost to the cotton plug. The strain used was known as No. 3 and was isolated some years ago. Using a 24-hour culture grown in these standard tubes, Gay and Claypole estimated that each slant yielded 1,400,000 million organisms. One third of such a culture (approximately 460,000 million bacteria) was injected (with one or two exceptions, which are noted) into the marginal ear vein. The introduction of this large number of living bacteria proves fatal to a small fraction of the animals used in an experiment, within

48 hours after inoculation, but those surviving usually live for months, or indefinitely. Of the rabbits inoculated with living cultures, 92 per cent. became carriers. None of the animals dying from 4-24 hours after inoculation are included in Table 1, but all such animals were examined and bile cultures made which in practically all cases showed large numbers of typhoid bacilli. Of the animals killed, 86 per cent. were chronic carriers.

The technic used was as follows: the animal was killed by a blow at the back of the neck. After making a small preliminary incision with aseptic precautions, sufficient blood was taken from the heart for culture and subsequent tests for agglutinating titer. After this, the fur having been closely clipped or shaved, and the abdominal surface thoroughly wet down with alcohol, a midline incision was made. Small pieces of the various organs to be cultured were removed with sterile instruments and placed in petri dishes which had previously been weighed. The spleen, liver, kidney, lymph nodes and bone marrow were used. Culture's were also made from the gallbladder and urine when possible. Rectal and duodenal plates were occasionally made, on litmus lactose and Endo mediums. The Petri dishes containing these sections were then re-weighed to determine the exact amount of tissue, and these pieces were macerated in small sterile hand mortars. Into each mortar was poured sufficient broth that each one-tenth gm. of tissue would be suspended in 1 c c of fluid. These broth-tissue mixtures were poured into test tubes and allowed to stand at room temperature for half an hour, to allow the larger masses to settle and produce a more uniform suspension. At the end of this time 1 c.c. of the tissue extract was added to approximately 9 of melted agar and the whole plated. Colonies were counted after 24 hours at 37 C.

It will be noted, in the case of normal rabbits, that within one-half an hour typhoid bacilli are found in all organs with especially large numbers in the blood, liver, spleen and bone marrow. No attempts have been made to determine how soon the bacillus appeared in the various organs, as this has been clearly worked out by Bull. The number of organisms in the blood rapidly decreases during the first few hours, but their persistence here and in the other organs seems to last until about the 14th day after inoculation. As has been noted by all writers on the subject of experimental carriers the typhoid bacillus was found in the bile long after it had disappeared from all other parts of the body, with the occasional exception of the bone marrow. I have so far made no attempt to trace its persistence in the bile beyond the 86th day.

From table 1 it may be seen that somewhat under 2 weeks must elapse before the organs of the normal animal become sterile. This is, of course, with the exception of the gallbladder. The blood, apparently, may clear up a short time before the organs, but this discrepancy in time is so short that it would seem very probable that the immune bodies arising in the serum, tissue cells or both, sterilize the blood and organs simultaneously, but that it is quite a while before the factor or

factors necessary for this phenomenon are available in sufficient quantity to rid the body of the invading micro-organism. Of further intertest is the fact that by the time agglutinins have reached a high titer, the animal is sterile.

TABLE 1

BACTERIAL COUNT FROM TISSUES OF NORMAL RABBITS INJECTED INTRAVENOUSLY WITH LIVING TYPHOID BACILLI

No.	Weight in Gm.	Time	Agglutinating Titer	Blood 1 c c	Bile ½ c c	Spleen	Liver	Kidney	Lymph	Marrow	Duodenal	Rectal	Urine
I	—	½ hr.	—	688	160	Inn	Inn	—	192	Inn	—	—	—
II	—	3 hr.	—	408	1,696	1,068	3,200	—	1,800	528+	—	—	—
904	3,700	6 hr.	—	54	18	960	800	—	2	Inn	—	—	—
902	4,300	24 hr.	—	20	Inn	Inn	750	—	2	Inn	—	—	—
24	3,500	24 hr.	—	30	28	Inn	Inn	400+	Inn	400+	—	—	—
Y	2,800	3 da.	1:100	34	Inn	800	240	—	60	Inn	—	—	—
17	2,250	3 da.	—	32	Inn	Inn	Inn	Inn	Inn	Inn	—	—	Inn—
22	1,850	7 da.	1:10.000	16	Inn	60	Inn	8	Inn	Inn	—	—	24
845	2,400	10 da.	1:5,120	0	Inn	16	6	—	150	Inn	—	—	—
367	1,500	10 da.	—	—	Inn	—	—	—	—	—	—	—	—
19	2,600	14 da.	1:6,400	0	Inn	0	0	0	0	0	—	—	0
478	2,250	15 da.	1:800	0	Inn	0	0	0	0	0	0	0	0
99	2,100	16 da.	1:3,200	0	Inn	0	0	0	0	0	—	—	0
20	2.950	17 da.	1:1,600	0	0	0	0	0	0	0	0	0	0
226	1,800	18 da.	—	—	Inn	—	—	—	—	—	—	—	—
X	2,200	21 da.	1:162,000	0	Inn	0	0	0	0	Inn	—	—	—
147	1,900	21 da.	—	—	Inn	0	0	0	0	0	—	0	0
434	2,700	23 da.	1:800	0	0	0	0	0	0	0	0	0	0
437	2.550	23 da.	1:400	0	Inn	0	0	0	0	0	0	0	0
96	2,050	30 da.	1:1,600	0	0	0	0	0	0	0	0	0	0
205	1,900	35 da.	1:1,600	0	Inn	0	0	0	0	0	0	0	0
143	1,700	42 da.	1:400	0	Inn	0	0	0	0	0	Inn	Inn	0
152	—	86 da.	—	—	Inn	—	—	—	—	—	—	—	—

Colonies per plate inoculated with 1 c c tissue-extract.
— = not cultured.
Inn = innumerable.
0 = sterile plate.
Nos. 143, 147, 152 = given ¼ blood agar slant instead of ⅛.

Immune Rabbits.—We find entirely different results in the destruction of the bacilli in highly immune animals. Cultures killed by heat were used for this immunization, each agar slant being washed off with 8 c.c. salt solution, and this emulsion heated to 60 C. for 30 minutes. This suspension of bacteria was tested for sterility before using.

One-sixteenth of a culture (½ c c) was the initial amount injected, this being followed by 1 c c on each of the next 2 successive days. After a lapse of 4 days another series of injections was given, usually in amounts of 1, 1½ and 2 c c. This was usually found sufficient to produce a serum of high agglutinating titer for typhoid bacilli. Further injections were given to those animals that had not been used for 2 weeks or more after the last series of injections, and the agglutinating power of whose serums had dropped appreciably.

These immunized rabbits presented, after inoculation, an entirely different appearance from the normal injected with living culture. Whereas, shortly after inoculation with living bacteria, the latter appeared sick and drooping, and within a period of 24 hours would lose as high as 400 grams in weight, the immunized rabbits remained lively and lost very little weight, giving little or no evidence of any physical inconvenience caused by the injection of such large numbers of living typhoid bacilli.

TABLE 2

BACTERIAL COUNT FROM TISSUES OF IMMUNIZED RABBITS INJECTED INTRAVENOUSLY WITH LIVING TYPHOID BACILLI

No.	Weight in Gm.	Time	Agglutinating Titer	Blood 1 c c	Bile ½ c c	Spleen	Liver	Kidney	Lymph	Marrow	Duodenal	Rectal	Urine
60	2,350	5 hr.	1:12,800	30	0	Inn	Inn	38	16	Inn	—	—	—
59	2,250	7 hr.	1:12,800	50	0	Inn	Inn	25	0	Inn	—	—	20
61	2,100	8 hr.	1:12,800	24	0	Inn	Inn	0	3	Inn	—	—	0
103	1,900	12 hr.	1:12,800	30	0	Inn	Inn	21	7	800	—	—	0
100	2,100	18 hr.	1:3,200	400	Inn	Inn	400	Inn	0	Inn	—	—	Inn
468	1,950	18 hr.	1:3,200	160	Inn	Inn	Inn	0	0	Inn	—	—	10
58	2,400	18 hr.	1:12,800	0	0	0	0	0	0	0	—	—	0
150	2,450	24 hr.	1:16,000	0	0	0	0	0	0	0	0	0	0
172	2,200	24 hr.	1:6,400	0	0	0	0	0	0	14	—	—	—
153	2,600	24 hr.	1:3,200	0	0	0	0	0	0	0	—	—	—
186	2,700	28 hr.	1:6,400	0	0	0	0	0	0	0	—	—	—

A comparison of table 2 with table 1 shows a remarkable difference between the normal and immune rabbits in their ability to rid themselves of the organisms injected. Whereas, in the normal rabbit, the typhoid bacilli can be isolated from the organs for at least 10 days after injection, immune animals are, but with occasional exceptions, sterile in less than 24 hours. An ability to quickly destroy large numbers of bacteria has been developed in the immune animals, a power to destroy in 24 hours or less the same number of organisms that it takes the normal rabbit almost 2 weeks to dispose of. Since it is evident that the rabbit carrier is analogous to the human, in general localization of the bacteria in the organs especially in the gallbladder, and their elimination with the feces, there is the possibility that the protection afforded human beings by vaccination may be compared with this resistance of immunized animals against living organisms. At any rate a study of this rapid destruction of typhoid bacilli by the immune rabbit may at least serve as an approach to an understanding of the mechanism of the immunity conferred in man by vaccination or recovery from disease.

EXPERIMENTS ON THE MECHANISM OF RESISTANCE

With this analogy in mind, a study of the mechanism of this immunity reaction was undertaken to determine, if possible, what factor or factors in the animal body are concerned in the development of the new property which makes its appearance after immunization.

Some years ago a set of experiments was carried out in this laboratory to test the protective value—in vivo—of typhoid immune rabbit serum. The M. L. D. of a certain laboratory strain (No. 3) of typhoid bacilli was determined by repeated intraperitoneal injection of varying dilutions of the culture

in mice. This having been determined, the protective value of the immune serum was tested, by injecting it into mice 6 hours before the injection of culture. Using a set amount of culture, various amounts of serum were tested, and it was found that as small a dose of serum as 0.01 c c protected mice against 2-3 M. L. D. of bacteria. Even when the injections of serum and bacilli were made simultaneously there was a certain amount of protection, although not as marked as when the serum was given 6 hours previous to the administration of the culture. Serum injected a short time after the culture was of no value, nor could any protecting power of normal serum be demonstrated no matter when injected. This would seem to indicate that new properties acquired by the serum are responsible for the protection of immune animals but subsequent experiments have shown that although the bacteriolytic power of the serum in vivo is very great, in vitro it has no effect on the organisms.

A repetition of the work by Bull, Buxton, Teague and Williams, and others on the comparative bactericidal properties of normal and immune serums in vitro, was carried out, with the results similar to those obtained by them. It was found that 1/10 c c or less of normal serum was usually sufficient to kill 1/10 c c of a 1:10,000 dilution of a 24-hour broth culture of B. typhosus. Repeated platings showed this to be about 1¼ million organisms. Immune serum, on the other hand, regardless of the agglutinating titer was nonbactericidal in amounts as large as 1 c c or more for the same number of organisms (Tables 3, 4).

TABLE 3

COMPARISON OF BACTERICIDAL POWER OF NORMAL AND IMMUNE SERUMS

					Subculture
1.	Fresh normal serum	0.5 c c	+	0.1 c c 1:10,000 dilution of culture	0
2.	Fresh normal serum	0.4 c c	+	0.1 c c 1:10,000 dilution of culture	0
3.	Fresh normal serum	0.3 c c	+	0.1 c c 1:10,000 dilution of culture	0
4.	Fresh normal serum	0.2 c c	+	0.1 c c 1:10,000 dilution of culture	0
5.	Fresh normal serum	0.1 c c	+	0.1 c c 1:10,000 dilution of culture	0
6.	Fresh normal serum	0.05 c c	+	0.1 c c 1:10,000 dilution of culture	+
7.	Fresh immune serum (153)	0.5 c c	+	0.1 c c 1:10,000 dilution of culture	+
8.	Fresh immune serum	0.4 c c	+	0.1 c c 1:10,000 dilution of culture	+
9.	Fresh immune serum	0.3 c c	+	0.1 c c 1:10,000 dilution of culture	+
10.	Fresh immune serum	0.2 c c	+	0.1 c c 1:10,000 dilution of culture	+
11.	Fresh immune serum	0.1 c c	+	0.1 c c 1:10,000 dilution of culture	+
12.	Fresh immune serum	0.05 c c	+	0.1 c c 1:10,000 dilution of culture	+

Agglutinating titer of No. 153 = 1:51,000. Subcultures made after 18 hours' incubation 0.1 c c of 1:10,000 dilution of culture = approximately 1¼ million organisms.

TABLE 4

CHANGE IN BACTERICIDAL POWER OF SERUM AFTER IMMUNIZATION

					Subculture
1.	No. 386 (before immunization)	0.5 c c	+	0.1 c c 1:10,000 dilution of culture	0
2.	No. 386 (before immunization)	0.4 c c	+	0.1 c c 1:10,000 dilution of culture	0
3.	No. 386 (before immunization)	0.3 c c	+	0.1 c c 1:10,000 dilution of culture	0
4.	No. 386 (before immunization)	0.2 c c	+	0.1 c c 1:10,000 dilution of culture	0
5.	No. 386 (before immunization)	0.1 c c	+	0.1 c c 1:10,000 dilution of culture	0
6.	No. 386 (before immunization)	0.05 c c	+	0.1 c c 1:10,000 dilution of culture	0
7.	No. 386 (after immunization)	0.5 c c	+	0.1 c c 1:100,000 dilution of culture	+
8.	No. 386 (after immunization)	0.4 c c	+	0.1 c c 1:100,000 dilution of culture	+
9.	No. 386 (after immunization)	0.3 c c	+	0.1 c c 1:100,000 dilution of culture	+
10.	No. 386 (after immunization)	0.2 c c	+	0.1 c c 1:100,000 dilution of culture	+
11.	No. 386 (after immunization)	0.1 c c	+	0.1 c c 1:100,000 dilution of culture	+
12.	No. 386 (after immunization)	0.05 c.c	+	0.1.c c 1:100,000 dilution of culture	+

Attempts to reactivate the immune serum were carried out as follows: Immune serum was heated to 56 C. for one-half hour. It was then reactivated by the addition of small amounts of fresh normal serum. If the amount of normal serum used in the reactivation was that known to be insufficient in itself to kill the standard number of bacilli used, there was no inhibition of growth. If, however, more than this amount of normal serum was used, no growth occurred, showing that the normal serum acted independently and was equally potent whether in immune serum or saline, and that any bactericidal properties present were due to the normal serum alone (Table 5).

TABLE 5

REACTIVATION OF IMMUNE SERUM

			Subculture
1.	Serum No. 153	0.5 c c + 0.1 c c culture (dilution 1:100,000)	+
2.	Serum No. 153	0.3 c c + 0.1 c c culture (dilution 1:100,000)	+
3.	Serum No. 153	0.1 c c + 0.1 c c culture (dilution 1:100,000)	+
4.	Serum No. 153 (56°)	0.4 c c + 0.1 c c culture (dilution 1:100,000) + 0.1 c c normal serum	0
5.	Serum No. 153	0.3 c c + 0.1 c c culture (dilution 1:100,000) + 0.1 c c normal serum	0
6.	Serum No. 153	0.2 c c + 0.1 c c culture (dilution 1:100,000) + 0.1 c c normal serum	0
7.	Serum No. 153	0.4 c c + 0.1 c c culture (dilution 1:100,000) + 0.05 c c normal serum	+
8.	Serum No. 153	0.3 c c + 0.1 c c culture (dilution 1:100,000) + 0.05 c c normal serum	+
9.	Serum No. 153	0.2 c c + 0.1 c c culture (dilution 1:100,000) + 0.05 c c normal serum	+
10.	Normal serum	0.3 c c + 0.1 c c culture (dilution 1:100,000)	0
11.	Normal serum	0.2 c c + 0.1 c c culture (dilution 1:100,000)	0
12.	Normal serum	0.1 c c + 0.1 c c culture (dilution 1:100,000)	0
13.	Normal serum	0.05 c c + 0.1 c c culture (dilution 1:100,000)	+

Tests were also made, using high dilutions of immune serum, the possibility being suggested that the agglutination of the bacteria protected them from the bactericidal action of the serum. However, no destruction of the bacteria could be found in dilutions as high as 1 to 1 million, using sera whose agglutinating titer were 1/6,400 or above (Table 6).

To test for possible leukocytic properties, whose addition might be necessary for the action of immune serum on bacteria, the following experiments were carried out. Intrapleural injections of 8 c c of broth were given to rabbits late in the afternoon. Early the next morning 6-8 c c more broth were injected into each pleural cavity and the rabbits killed by exsanguination 2 hours later. The admixture of sterile sand to the broth to increase by irritation the leukocytic output, was found of no particular advantage. Eight to 10 c c of creamery fluid could be recovered from each pleural cavity, which was found to contain enormous numbers of leukocytes, mainly polymorphonuclears. This was collected in 2-3 c c of 1 per cent. sodium citrate, to prevent clotting, and the cells sedimented by gentle trifugalization. The supernatant fluid was then poured off and the suspension restored to its original volume with normal salt solution. Smears showed that these leukocytes had not suffered by this treatment.

This leukocytic suspension was then added to different dilutions of immune serum and living typhoid bacilli, to demonstrate, if possible, any bacteriolytic effect of the serum-leukocyte combination. But no inhibition of the bacterial growth could be demonstrated, nor have the leukocytes alone any lytic effect on the bacteria. Slides made at 20 and 40 minutes after the addition of the bacteria to the leukocytes and serum showed no phagocytosis. However, this would probably have been difficult to demonstrate because of the small number of bacteria introduced (Table 6).

TABLE 6

IMMUNE SERUM AND LEUKOCYTES

							Subculture
Immune Serum (fresh)—							
1.	No. 386 0.3 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:10,000)		+
2.	No. 386 0.2 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:10,000)		+
3.	No. 386 0.1 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:10,000)		+
4.	No. 386 0.01 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:10,000)		+
5.	No. 386 0.001 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:10,000)		+
6.	No. 386 0.0001 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:10,000)		+
7.	No. 386 0.00001 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:10,000)		+
8.	No. 315 0.3 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:10 000)		+
9.	No. 315 0.2 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:10,000)		+
10.	No. 315 0.1 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:10,000)		+
11.	No. 315 0.01 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:10,000)		+
12.	No. 315 0.001 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:10,000)		+
13.	No. 315 0.0001 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:10,000)		+
14.	No. 315 0.00001 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:10,000)		+
15.	No. 386 0.3 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:100,000)		+
16.	No. 386 0.3 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:100,000.000)		+
17.	No. 315 0.3 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:100.000)		+
18.	No. 315 0.3 c c	+	leukocytes 0.2 c c	+	0.1 c c culture (1:100,000,000)		+
Normal Serum (fresh)—							
19.	0.5 c c	+	0.1 c c culture (1:10 000)				0
20.	0.4 c c	+	0.1 c c culture (1:10 000)				0
21.	0.3 c c	+	0.1 c c culture (1:10 000)				0
22.	0.2 c c	+	0.1 c c culture (1:10,000)				0
23.	0.1 c c	+	0.1 c c culture (1:10,000)				0
24.	0.05 c c	+	0.1 c c culture (1:10,000)				+
Immune Serum (fresh)—							
25.	No. 386 0.4 c c	+	0.1 c c culture (1:10,000)				+
26.	No. 386 0.3 c c	+	0.1 c c culture (1:10 000)				+
27.	No. 386 0.2 c c	+	0.1 c c cultrue (1:10,000)				+
28.	No. 386 0.1 c c	+	0.1 c c culture (1:10,000)				+
29.	No. 386 0.05 c c	+	0.1 c c culture (1:10.000)				+
30.	No. 386 0.01 c c	+	0.1 c c culture (1:10.000)				+
31.	No. 386 0.001 c c	+	0.1 c c culture (1:10 000)				+
32.	No. 386 0.0001 c c	+	0.1 c c culture (1:10,000)				+
33.	No. 386 0.00001 c c	+	0.1 c c culture (1:10,000)				+
34.	No. 315 0.4 c c	+	0.1 c c culture (1:10.000)				+
35.	No. 315 0.3 c c	+	0.1 c c culture (1:10,000)				+
36.	No. 315 0.2 c c	+	0.1 c c culture (1:10,000)				+
37.	No. 315 0.1 c c	+	0.1 c c culture (1:10,000)				+
38.	No. 315 0.05 c c	+	0.1 c c culture (1:10.000)				+
39.	No. 315 0.01 c c	+	0.1 c c culture (1:10 000)				+
40.	No. 315 0.001 c c	+	0.1 c c culture (1:10,000)				+
41.	No. 315 0.0001 c c	+	0.1 c c culture (1:10,000)				+
42.	No. 315 0.00001 c c	+	0.1 c c culture (1:10,000)				+
43.	No. 369 0.4 c c	+	0.1 c c culture (1:10,000)				+
44.	No. 369 0.3 c c	+	0.1 c c culture (1:10,000)				+
45.	No. 369 0.2 c c	+	0.1 c c culture (1:10,000)				+
46.	No. 369 0.1 c c	+	0.1 c c culture (1:10,000)				+
47.	No. 369 0.05 c c	+	0.1 c c culture (1:10,000)				+
48.	No. 369 0.01 c c	+	0.1 c c culture (1:10.000)				+
49.	No. 369 0.001 c c	+	0.1 c c culture (1:10.000)				+
50.	No. 369 0.0001 c c	+	0.1 c c culture (1:10,000)				+
51.	Leukocytes 0.2 c c	+	0.1 c c culture (1:10,000)				+

To test for the possible activity of the tissue cells in the rapid destruction of typhoid bacilli in the immune animal, the tissues of such animals were tested with respect to their destruction of the bacteria brought in contact with them. Highly immune rabbits were exsanguinated by bleeding from the carotid. Pieces of organs were coarsely ground in mortars with the aid of a small amount of sand, and to these were added a comparatively small number of typhoid bacilli (1/10 c c of a 1:500,000 dilution of broth culture). After incubation for 18 hours at 37 C. plates were streaked. All gave a good growth of typhoid bacilli. Attempts were also made to bring about bacteriolysis by the addition of homologous immune serum to these tissue-bacteria mixtures, but no inhibition of multiplication of the bacilli could be demonstrated (Table 7).

TABLE 7

IMMUNE SERUM AND TISSUES

		Culture
1.	Liver (cut surface) + 0.2 c c immune serum + 0.1 1:500.000 dil. culture	+
2.	Spleen (cut surface) + 0.2 c c immune serum + 0.1 1:500,000 dil. culture	+
3.	Kidney (cut surface) + 0.2 c c immune serum + 0.1 1:500,000 dil. culture	+
4.	Lymph node (cut surface) + 0.2 c c immune serum + 0.1 1:500,000 dil. culture	+
5.	Bone marrow (cut surface) + 0.2 c c immune serum + 0.1 1:500,000 dil. culture	+
6.	Liver (macerated) + 0.2 c c immune serum + 0.1 1:500,000 dil. culture	+
7.	Spleen (macerated) + 0.2 c c immune serum + 0.1 1:500,000 dil. culture	+
8.	Kidney (macerated) + 0.2 c c immune serum + 0.1 1:500,000 dil. culture	+
9.	Lymph node (macerated) + 0.2 c c immune serum + 0.1 1:500.000 dil. culture	+
10.	Bone marrow (macerated) + 0.2 c c immune serum + 0.1 1:500,000 dil. culture	+
11.	Liver (cut surface) + 0.1 1:500,000 dil. culture	+
12.	Spleen (cut surface) + 0.1 1:500,000 dil. culture	+
13.	Kidney (cut surface) + 0.1 1:500,000 dil. culture	+
14.	Lymph node (cut surface) + 0.1 1:500,000 dil. culture	+
15.	Bone marrow (cut surface) + 0.1 1:500,000 dil. culture	+
16.	Liver (macerated) + 0.1 1:500.000 dil. cultrue	+
17.	Spleen (macerated) + 0.1 1:500,000 dil. culture	+
18.	Kidney (macerated) + 0.1 1:500,000 dil. culture	+
19.	Lymph node (macerated) + 0.1 1:500 000 dil. culture	+
20.	Bone marrow (macerated) + 0.1 1:500.000 dil. culture	+
21.	0.2 c c immune serum + 0.1 1:500,000 dil. culture	+

Unmacerated cut sections of organs were also inoculated to test for any possible action of the tissue cells that might have been destroyed by grinding. But there was no bacteriolysis, either when bacteria alone were used, or when immune serum was added. In fact, all these experiments showed as rapid a growth of the organism as there would be on any favorable culture medium.

It seems that we are confronted with some rather paradoxical facts concerning the mechanism of bacteriolysis in the body of the immune animal, namely:

1. Typhoid bacilli disappear more quickly from the organs of immune animals than from normal animals.

2. Macerated organs, from immune animals, cut sections, or their extracts are not bactericidal even on the addition of fresh immune serum.

3. Typhoid immune serum is nonbactericidal for typhoid bacilli in vitro.